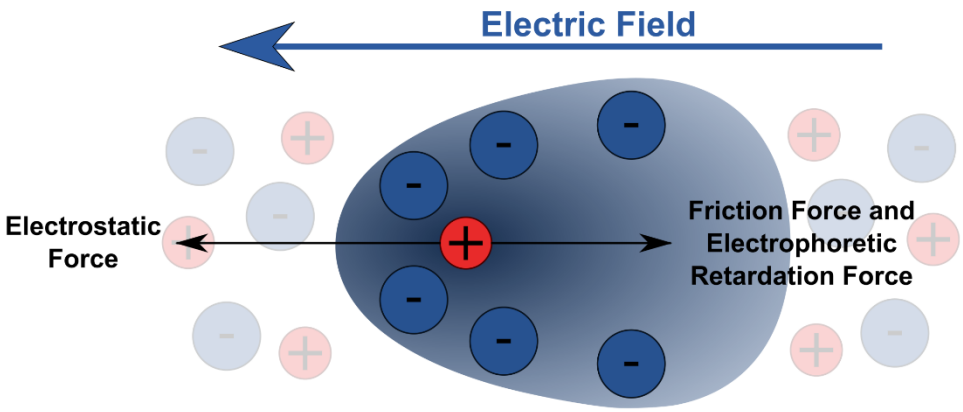
Electrophoresis

Electrophoresis

Principles of Separation

- Electrophoresis is a separation technique based on the movement of charged particles (ions, proteins, nucleic acids, or drug molecules) in an electric field through a supporting medium (paper or gel).



Core Principle:

- When an electric field is applied:
  - **Charged molecules migrate toward the electrode of opposite charge:**
    - Cations → move to the cathode (-)
    - Anions → move to the anode (+)
  - **The rate of migration depends on:**
    - Net charge of the molecule
    - Size and shape
    - Electric field strength
    - Viscosity and composition of the supporting medium
    - pH of the buffer (affects ionization of molecules)
- This results in the separation of different components in a mixture.

Electrophoresis is particularly useful for:

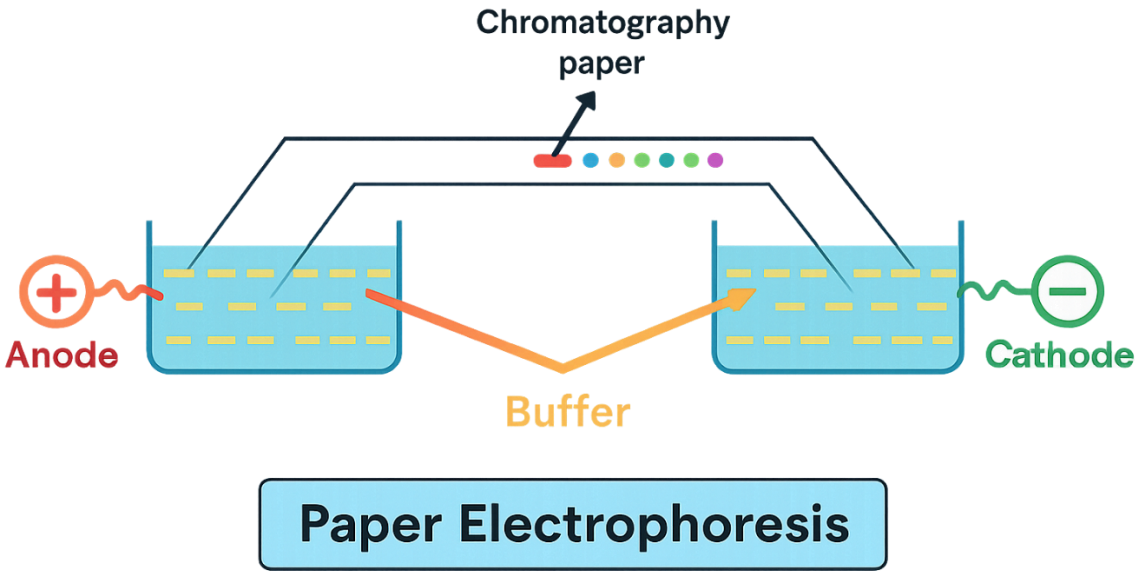
- Charged drug molecules
- Biological macromolecules (e.g., proteins, peptides, nucleic acids)
- Complex drug products (e.g., vaccines, biosimilars)

Equipment for Electrophoresis

- Electrophoresis setups may vary based on the medium used.
- Two common types in pharmaceutical applications are paper electrophoresis and gel electrophoresis.

Paper Electrophoresis

Components and Setup:



1. Support Medium:

- Strips of filter paper (e.g., Whatman No. 1) soaked in a buffer solution (e.g., phosphate, acetate).

2. Sample Application:

- The drug or biomolecule solution is spotted in the center or near one end of the paper strip.

3. Buffer Chamber:

- Two buffer reservoirs connected to electrodes.
- The paper strip bridges the two chambers, ensuring continuous **buffer contact**.

4. Electrodes and Power Supply:

- A **DC power supply** generates an electric field across the strip (typically 100–400 V).
- The molecules migrate over a defined time (e.g., 1–2 hours).

5. Visualization:

- After development, the paper is dried and **stained** using specific reagents:
  - Ninhydrin for amino acids
  - Silver stain for proteins
  - UV light for nucleotides

Advantages:

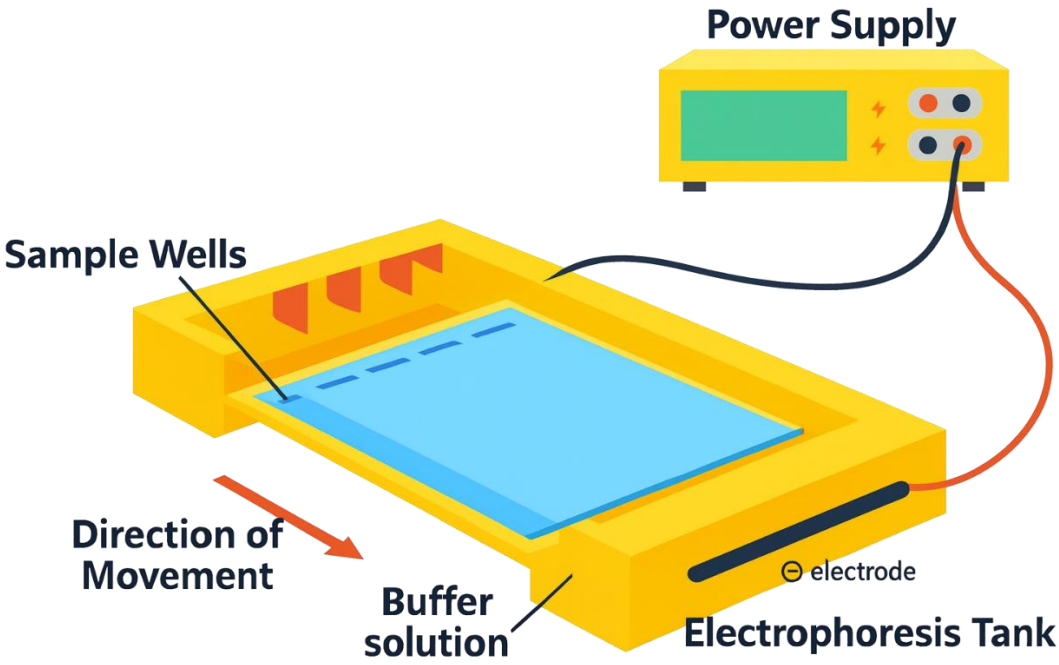
- Simple and low-cost
- Good for small, ionizable drug molecules

Limitations:

- Low resolution
- Not suitable for large biomolecules
- Manual detection and analysis

Gel Electrophoresis

- Used for proteins, peptides, nucleic acids, and charged drug aggregates.



Types of Gels:

- **Agarose gel**: For large molecules (e.g., DNA, RNA)
- **Polyacrylamide gel** (PAGE): For proteins, peptides, small nucleic acids

Equipment Components:

1. **Gel Casting Tray:**
   - Used to prepare and solidify the gel with wells for sample loading.
2. **Buffer Solution:**
   - Maintains **pH** and conductivity (e.g., Tris-acetate, Tris-glycine buffer).
3. **Electrophoresis Tank:**
   - Holds the gel and buffer. Electrodes are connected at both ends.
4. **Sample Loading:**
   - Drug samples or biomolecules mixed with loading dye are added to wells using micropipettes.

5. Power Supply:

- Provides a constant electric field (50–300 V), allowing separation by size and charge.

6. Detection/Staining:

- Coomassie Brilliant Blue, Silver stain, or Ethidium bromide for visualization.
- Densitometry or gel documentation systems for quantitative analysis.

Applications of Electrophoresis

A. Purity and Identity Testing of Biomolecules

- Monoclonal antibodies, vaccines, insulin, interferons
- Separation and identification of subunits, aggregates, or fragments
- Used during development, quality control, and batch release

B. Characterization of Peptides and Proteins

- Molecular weight determination using SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis)
- Isoelectric focusing (IEF) determines isoelectric point (pI) of proteins.
- Example: Characterization of recombinant human insulin formulations

C. Nucleic Acid Analysis

- Agarose gel electrophoresis used in gene therapy product QC
- Used to analyze plasmids, siRNA, mRNA vaccines

D. Detection of Impurities

- Identifies charged impurities or degradation products not visible by HPLC or TLC.
- For example: protein oxidation or deamidation

E. Separation of Charged Drugs

- Certain small drug molecules (e.g., sulfonamides, phenothiazines) can be separated by electrophoresis in forensic and pharmacokinetic studies.

F. Drug-Excipient Interaction Studies

- Changes in mobility patterns indicate potential interactions or degradation.

G. Bioequivalence and Biosimilarity Studies

- Compares biosimilar products to reference biologics using gel electrophoresis profiles.

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